OLIGONUCLEOTIDES HAVING A-DNA FORM AND B-DNA FORM CONFORMATIONAL GEOMETRY

CROSS-REFERENCE OF RELATED APPLICATIONS

[0001] This Application is a continuation-in-part of 09/303,586, filed May 3, 1999 and of 08/936,166, filed September 23, 1997. Application Serial No. 08/936, 166 is a divisional of 07/835,932, filed March 5, 1992, now U.S. Patent No. 5,670,633, which derives from International Patent Application Serial No. PCT/US91/05720, filed August 12, 1991 and published as WO 92/03568 on March 5, 1992. Each of the foregoing applications is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to oligonucleotides that have both A-form and B-form conformational geometry and methods of using such oligonucleotides. The oligonucleotides of the invention are useful in therapeutic and investigative purposes. More specifically, the present invention is directed to oligonucleotides having particular modifications that will increase affinity and nuclease resistance while concurrently serving as substrates for RNase H when bound to a target RNA strand.

BACKGROUND OF THE INVENTION

[0003] It is well known that most of the bodily states in mammals, including most disease

states, are affected by proteins. Classical therapeutic modes have generally focused on interactions with such proteins in an effort to moderate their disease-causing or disease-potentiating functions. However, recently, attempts have been made to moderate the actual production of such proteins by interactions with molecules that direct their synthesis, such as intracellular RNA. By interfering with the production of proteins, maximum therapeutic effect and minimal side effects may be realized. It is the general object of such therapeutic approaches to interfere with or otherwise modulate gene expression leading to undesired protein formation.

[0004] One method for inhibiting specific gene expression is the use of oligonucleotides. Oligonucleotides are now accepted as therapeutic agents. A first such oligonucleotide has been approved for human therapeutic use by the FDA and is available in commercial marketplace.

[0005] Oligonucleotides are known to hybridize to single-stranded DNA or RNA molecules. Hybridization is the sequence-specific base pair hydrogen bonding of nucleobases of the oligonucleotide to the nucleobases of the target DNA or RNA molecule. Such nucleobase pairs are said to be complementary to one another. The concept of inhibiting gene expression through the use of sequence-specific binding of oligonucleotides to target RNA sequences, also known as antisense inhibition, has been demonstrated in a variety of systems, including living cells (for example see: Wagner *et al.*, Science (1993) 260: 1510-1513; Milligan *et al.*, *J. Med. Chem.*, (1993) 36:1923-37; Uhlmann *et al.*, *Chem. Reviews*, (1990) 90:543-584; Stein *et al.*, *Cancer Res.*, (1988) 48:2659-2668).

[0006] The events that provide the disruption of the nucleic acid function by antisense oligonucleotides (Cohen in *Oligonucleotides: Antisense Inhibitors of Gene Expression*, (1989) CRC Press, Inc., Boca Raton, FL) are thought to be of two types. The first, hybridization arrest, denotes

the terminating event in which the oligonucleotide inhibitor binds to the target nucleic acid and thus prevents, by simple steric hindrance, the binding of essential proteins, most often ribosomes, to the nucleic acid. Methyl phosphonate oligonucleotides: Miller, P.S. and Ts'O, P.O.P. (1987) *Anti-Cancer Drug Design*, 2:117-128, and α-anomer oligonucleotides are the two most extensively studied antisense agents which are thought to disrupt nucleic acid function by hybridization arrest.

[0007] The second type of terminating event for antisense oligonucleotides involves the enzymatic cleavage of the targeted RNA by intracellular RNase H. A 2'-deoxyribofuranosyl oligonucleotide or oligonucleotide analog hybridizes with the targeted RNA and this duplex activates the RNase H enzyme to cleave the RNA strand, thus destroying the normal function of the RNA. Phosphorothioate oligonucleotides are probably the most prominent example of an antisense agent that operates by this type of antisense terminating event.

[0008] Oligonucleotides may also bind to duplex nucleic acids to form triplex complexes in a sequence specific manner via Hoogsteen base pairing (Beal *et al.*, *Science*, (1991) 251:1360-1363; Young *et al.*, *Proc. Natl. Acad. Sci.* (1991) 88:10023-10026). Both antisense and triple helix therapeutic strategies are directed towards nucleic acid sequences that are involved in or responsible for establishing or maintaining disease conditions. Such target nucleic acid sequences may be found in the genomes of pathogenic organisms including bacteria, yeasts, fungi, protozoa, parasites, viruses, or may be endogenous in nature. By hybridizing to and modifying the expression of a gene important for the establishment, maintenance or elimination of a disease condition, the corresponding condition may be cured, prevented or ameliorated.

[0009] In determining the extent of hybridization of an oligonucleotide to a complementary